CASWELL FILE

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

SEP -2 1986

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MEMORANDUM

SUBJECT: Isofenphos Subchronic Delayed Neurotoxicity Study; Mobay report # 90231; Caswell # 447AB; Project # 128

TO: William H. Miller
Product Manager (16)
Registration Division (TS-767C)

FROM: James N. Rowe. Ph.D.
Section V, Toxicology Branch
Hazard Evalution Division/HED (TS-769C)

James N. Rowe 8/29/86

THRU: Laurence D. Chitlik, D.A.B.T.
Section Head, Section V
Toxicology Branch/HED (TS-769C)
and
Theodore Farber, Ph.D.
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769C)

9/2/86

ACTION: Review isofenphos subchronic neurotoxicity study entitled, " Study for Subchronic Neurotoxicity (90-Day Study with Chickens)"; submitted by Mobay Chemical Corporation as study # 90231; A 258563, 073466; Cas.# 447AB; EPA I.D. 3125-326

RECOMMENDATIONS:

Based on the significant depressions in body weight and cholinesterase activity at the high dose, without any evidence of neuronal degeneration--as determined by no change in gait at any dose level during the course of the study and no apparent histopathological changes at the high dose level--isofenphos does not appear to produce delayed neurotoxicity. The tentative delayed neurotoxicity NOEL is set at >2 mg/kg (HDT). The slight, consistent nerve degeneration observed in the spinal cord of the vehicle control and high dose groups is stated in the report to be the result "of the conventional husbandry of these chickens, which had been commercially used before the study". The reviewer is concerned that these findings could mask any subtle effect of isofenphos. Therefore, it is requested that additional data be submitted to substantiate that this is a normal background neuropathological change in chickens.

This study is designated as Core Supplementary data. It may be upgraded upon submission and approval of the requested additional data.

DATA EVALUATION RECORD

STUDY/ACTION TYPE: Subchronic delayed neurotoxicity in White Leghorn hens

CHEMICAL: Isofenphos: 1-methylethyl 2-[[ethoxy[(1-methylethyl)amino] phosphinothioyl] oxy]benzoate

TEST MATERIAL: Technical Oftanol; batch no. 0005281 (Mobay Chemical Corporation); purity 92.5% (communication of Mobay Chemical Corporation of 7/16/84); test compound was refrigerated at a temperature of 8° - 13°C

STUDY I.D.:

1. Title: " Study for Subchronic Neurotoxicity (90-Day Study with Chickens)"

2. Laboratory: Bayer AG, Institute of Toxicology, Wuppertal-Elberfeld, West Germany

3. Sponsor: Mobay Chemical Corporation, Agricultural Chemicals Division, Kansas City, Mo. 64120

4. Study #: T 7017 964

5. Date of Report: 5/13/85

6. Study Director: Dr. W. Flucke

7. Caswell # 447AB, Accession 258563 073466; EPA ID # 3125-326

METHODS:

A photocopy of the methods section is appended. The following comments are noted:

1. The age of the hens was given as 15-20 months old which is not optimum for testing for delayed neurotoxicity, the EPA recommended age being between 8-14 months of age.

2. The solubility of Isofenphos in water is approximately 20 ppm, therefore the homogeneity of the material in an aqueous vehicle was of concern. The report presented data (pgs. 45-48) on the compound's homogeneity in 2% Cremophor/water indicating that at various concentrations(0.001 to 1.0 %) the material was of an adequate homogeneous nature (82 to 98% of specified value).

3. The pilot study (Section 3.4 of methods) was discussed but not submitted.

RESULTS:

1. Clinical signs/symptoms; mortality

In general, there were no unusual clinical observations except for one animal (no. 19) in the vehicle control which exhibited a waddling gait from day 14 onwards and which was sacrificed on day 30. In addition, a high dose animal (no.

44) was reported as apathetic with reduced mobility on day 4 and dying on day 5 of the test.

2. Body weights : Table 1 (see below)

Isofenphos statistically significantly reduced ($p<0.05$ and/or $p<0.01$) body weights when compared against the vehicle controls (1.50 kg/high dose vs 1.73 kg/ control) by the end of week 1 of administration and this depression in body weight continued through week 13 of the study. The positive delayed neurotoxic control (TOCP) also reduced the mean body weights from week 11 on, although the depression in weight was not statistically significant.

Table 1: Body weight means (kg)

Dose group	W0	W1	W2	W3	W4	W5 ...	W11	W12	W13
I(0 mg/kg)	1.87	1.81	1.80	1.79	1.85	1.86	1.66	1.63	1.60
II(0 mg/kg: vehicle)	1.79	1.73	1.74	1.80	1.84	1.82	1.79	1.74	1.70
V(2 mg/kg)	1.72	1.50*	1.51†	1.55†	1.58†	1.63*	1.52†	1.50*	1.55
VI(TOCP)	1.79	1.74	1.73	1.74	1.70	1.70	1.57	1.54	1.54

W= week on test; *significantly different from vehicle control($p<0.05$); † significantly different from vehicle control ($p<0.01$)

3. Forced motor activity: shooing and ladder climbing

Shooing: A slightly abnormal gait (grade no.1) or ataxia (grade no. 2) was observed only during week 7 or 8 in all the isofenphos groups which was not dose-related and the animals did not continue to exhibit this effect on mobility. The authors reported that this was due to an over vigorous shooing of the hens dosed with isofenphos. The TOCP control group showed decreased or aberrant mobility by the beginning of week 4 which grew progressively more pronounced through week 13 (grade 1 effect/2 animals for week 4; primarily grade 2 by week 13 in all animals). One animal in the positive control group had severe ataxia by week 9 which continued throughout the rest of the test period (animal no. 52).

Ladder climbing: No statistically significant variations in the forced ladder climbing times among any of the groups was reported. Chicken no. 52 of the TOCP group showed severe ataxia from week 9 onwards and was reported to repeatedly refuse to climb the ladder due to severe impairment of motor coordination.

4. Cholinesterase activities: Table 2 (see below)

Cholinesterase activities in blood plasma, erythrocytes (RBCs) and whole blood were examined. Plasma cholinesterase (pseudocholinesterase) was statistically significantly inhibited on day 26 of sampling at the 1 and 2 mg/kg doses compared to the vehicle control (0.52 U/ml = mid, 0.39 U/ml = high vs 1.10 U/ml, vehicle control).

There was also depressed cholinesterase activities in the RBCs (true cholinesterase) at day 26 of test (statistically significant only at high dose) as well as the depressed cholinesterase in whole blood at both day 55 (statistically significant at both dose levels) and day 83 on test (lower but not statistically signficant at both doses). The TOCP controls showed somewhat lower plasma and RBC cholinesterase activities at day 26 than the vehicle controls (0.76 and 0.33 U/ml in treated, respectively, vs. 1.10 and 0.38 U/ml, respectively in controls). No apparent effect on cholinesterase activity in whole blood in the positive control group was evident.

Table 2: mean cholinesterase activity (U/ml)

Dose group	Plasma(day 26)	Erythrocyte(day 26)	Whole Blood		
			d0	d55	d83
I(0 mg/kg)	0.78	0.45	0.67	0.63	0.55
II(0 mg/kg: vehicle)	1.10	0.38	0.72	0.61	0.55
III(0.25 mg/kg)	0.82	0.36	0.73	0.59	0.58
IV(1.00 mg/kg)	0.52†	0.33	0.73	0.46*	0.51
V(2 mg/kg)	0.39*	0.29*	0.65	0.39*	0.44
VI(TOCP)	0.76	0.33	0.75	0.68	0.65

d= day of test; * significantly different from vehicle control($p<0.05$); † significantly different from vehicle control ($p<0.01$)

5. Gross necropsy/ histopathology

Gross necropsy: No differences in gross pathology were noted when the controls were compared against the treatment groups.

Histopathology: The vehicle control, high dose and positive control groups were examined for microscopic findings (tables on pages 39-41 of the report).

There were no unusual differences in the histology for the vehicle control group and the high dose group. Both groups showed a consistent lympho-histiocytic infiltration in the sciatic nerve, lumbar and medulla oblongata/cerebellar nerve sections which was suggested by the study author to be a normal observation in commercially used chickens. Generally slight degeneration was observed in both the control and high dose group thoracic (9/10 vs 9/10, respectively) and cervical (7/10 vs 7/10, respectively) nerve sections. One animal in the high dose group had autolyzed nerve tissue which prevented meaningful histological examination.

In contrast to the high dose group, the TOCP positive control had generally moderate to severe nerve degeneration in the lumbar, thoracic and cervical nerves of the spinal cord as well as slight to moderate nerve degeneration in the medulla oblongata/cerebellum in all animals. This is in contrast to the previously

mentioned slight nerve degeneration observed in the cervical and lumbar nerves of the vehicle control animals. Lympho-histiocytic infiltration of the sciatic nerve was also a consistent finding in the positive controls.

CONCLUSIONS/RECOMMENDATIONS:

The mid and high doses of isofenphos administered were adequate to induce some systemic toxicity. Isofenphos produced a statistically significant depression in mean body weights at the high dose level (2 mg/kg) in week 1 on test and this depression continued throughout the period of test compound administration. The test compound also produced a statistically significant inhibition in plasma cholinesterase on day 26 of blood sampling in both the mid (1 mg/kg) and high dose groups (2 mg/kg) as well as depressed RBC cholinesterase activity at day 26 and depressed cholinesterase in whole blood at both day 55 (statistically significant at both dose levels) and day 83 on test (not statistically signficant at both doses).

In contrast to the positive control (TOCP), which elicited ataxia in all animals by week 13 on test, no isofenphos-related effects on motor coordination were observed during forced motor activity (shooing). No compound-related histopathology was observed in the high dose group as compared to the vehicle control, although there was some evidence of slight nerve degeneration of the thoracic and cervical nerve fibers in both the control and high dose animals. The positive control produced the expected neural degeneration indicative of its delayed neurotoxicity in both the peripheral and central neurons. This indicates the responsiveness of the test animals to a delayed neurotoxicant.

Based on the significant depressions in body weight and cholinesterase activity at the high dose, without any evidence of neuronal degeneration--as determined by no change in gait at any dose level during the course of the study and no apparent histopathological changes at the high dose level--isofenphos does not appear to produce delayed neurotoxicity. The tentative delayed neurotoxicity NOEL is set at >2 mg/kg (HDT). The slight, consistent nerve degeneration observed in the spinal cord of the vehicle control and high dose groups is stated in the report to be the result "of the conventional husbandry of these chickens, which had been commercially used before the study". The reviewer is concerned that these findings could mask any subtle effect of isofenphos. Therefore, it is requested that additional data be submitted to substantiate that this is a normal background neuropathological change in chickens.

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